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TARALP, ALPAY
SAYERS, SEHRA

<120> CIRCULAR RECOMBINANT PLASMID DNA CONSTRUCTS AND THEIR PROTEIN PRODUCTS, METHODS OF PREPARATION AND IMMOBILISATION OF PROTEINS ON SUPPORT

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<140> 10/550226
<141> 2005-09-20

<150> PCT/TR2003/000019
<151> 2003-03-20

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gtt caa tgc ttt tcc cgt tat ccg gat cat atg aaa cgg cat gac ttt 537
Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg His Asp Phe
70 75 80

ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa cgc act ata tct 585
Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Ser
85 90 95 100

ttc aaa gat gac ggg aac tac aag acg cgt gct gaa gtc aag ttt gaa 633
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
105 110 115

ggt gat acc ctt gtt aat cgt atc gag tta aaa ggt att gat ttt aaa 681
Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
120 125 130

gaa gat gga aac att ctc gga cac aaa ctc gag tac aac tat aac tca 729
Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser
135 140 145

cac aat gta tac atc acg gca gac aaa caa aag aat gga atc aaa gct 777
His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
150 155 160

aac ttc aaa att cgc cac aac att gaa gat gga tcc gtt caa cta gca 825
Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala
165 170 175 180

gac cat tat caa caa aat act cca att ggc gat ggc cct gtc ctt tta 873
Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
185 190 195

cca gac aac cat tac ctg tcg aca caa tct gcc ctt tcg aaa gat ccc 921
Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro
200 205 210

aac gaa aag cgt gac cac atg gtc ctt ctt gag ttt gta act gct gct 969
Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
215 220 225

ggg att aca cat ggc atg gat gag ctc tac aaa taa tga att cca 1014
Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys Ile Pro
230 235 240

actgagcgcc ggtcgctacc attaccaact tgtctggtgt caaaaataat aggcctacta 1074

gtcggccgta cgggcccttt cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac 1134

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cctgattctg tggataaccg tattaccgcc tttgagtgag ctgataccgc tcgccgcagc 3294

cgaacgaccg agcgcagcga gtcagtgagc gaggaagcgg aag 3337

<210> 5
<211> 239
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 5

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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145 150 155 160

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
210 215 220

Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 6
<211> 6069
<212> DNA
<213> Artificial sequence

<220>
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<223> pETM-GFP-Imm plasmid with Hisx6 tag and A. victoria GFP gene

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Met Lys His His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
1 5 10 15

act act gag aat ctt tat ttt cag ggc gcc atg gga ggc acg gta ccg 96
Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
20 25 30

gta gaa aaa atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca 144
Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
35 40 45

att ctt gtt gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc 192
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
50 55 60

agt gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa 240
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65 70 75 80

ttt att tgc act act gga aaa cta cct gtt cca tgg cca aca ctt gtc 288
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
85 90 95

act act ttc tct tat ggt gtt caa tgc ttt tcc cgt tat ccg gat cat 336
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
100 105 110

atg aaa cgg cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta 384
Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
115 120 125

cag gaa cgc act ata tct ttc aaa gat gac ggg aac tac aag acg cgt 432
Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
130 135 140

gct gaa gtc aag ttt gaa ggt gat acc ctt gtt aat cgt atc gag tta 480
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145 150 155 160

aaa ggt att gat ttt aaa gaa gat gga aac att ctc gga cac aaa ctc 528
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
165 170 175

gag tac aac tat aac tca cac aat gta tac atc acg gca gac aaa caa 576
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
180 185 190

aag aat gga atc aaa gct aac ttc aaa att cgc cac aac att gaa gat 624
Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
195 200 205

gga tcc gtt caa cta gca gac cat tat caa caa aat act cca att ggc 672
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
210 215 220

gat ggc cct gtc ctt tta cca gac aac cat tac ctg tcg aca caa tct 720
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225 230 235 240

gcc ctt tcg aaa gat ccc aac gaa aag cgt gac cac atg gtc ctt ctt 768
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
245 250 255

gag ttt gta act gct gct ggg att aca cat ggc atg gat gag ctc cgt 816
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
260 265 270

cga caa gct tgc ggc cgc act cga gca cca cca cca cca ctg aga 864
Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Pro Leu Arg
275 280 285

tcc ggc tgc taa caaagcccga aaggaagctg agttggctgc tgccaccgct 916
Ser Gly Cys
290

gagcaataac tagcataacc ccttggggcc tctaaacggg tcttgagggg ttttttgctg 976

aaaggaggaa ctatatccgg attggcgaat gggacgcgcc ctgtagcggc gcattaagcg 1036

cggcgggtgt ggtggttacg cgcagcgtga ccgctacact tgccagcgcc ctagcgcccg 1096

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gatcgagatc tcgatcccgc gaaattaata cgactcacta taggggaatt gtgagcggat 6016

aacaattccc ctctagaaat aattttgatt taactttaag aaggagatat acc 6069

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<211> 291
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Construct

<400> 7

Met Lys His His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
1 5 10 15

Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
20 25 30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
35 40 45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
50 55 60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65 70 75 80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
85 90 95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
100 105 110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
115 120 125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
130 135 140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145 150 155 160

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
165 170 175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
180 185 190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
195 200 205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
210 215 220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225 230 235 240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
245 250 255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
260 265 270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Pro Leu Arg
275 280 285

Ser Gly Cys
290

<210> 8
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Frame adapter used for prevention of frameshift mutation as a result of plasmid modification

<400> 8
catgggaggc acggtac 17

<210> 9
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> Peptide design based on size and flexibility to act as a linker between the tag and GFP protein segments

<400> 9

Met Gly Gly Thr Val
1 5

<210> 10
<211> 6
<212> PRT

<213> Artificial sequence

<220>
<223> Peptide design based on charge and shape to bind the expressed protein to a suitably interactive surface

<400> 10

His His His His His His
1 5

<210> 11
<211> 238
<212> PRT
<213> Aequorea victora

<220>
<221> MISC_FEATURE
<223> Green fluorescent peptide coded by pGFPuv plasmid, permitting easy visualisation and quantification based on fluorescence properties

<400> 11

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 12
<211> 291
<212> PRT
<213> Artificial sequence

<220>
<223> Completed peptide based on Hisx6 tag, short physically flexible linker and green fluorescent protein coded by pETM-GFP-Imm to impart fluorescence properties, allowing easy immobilisation with retention of bioactivity, visualisation and quantification

<400> 12

Met Lys His His His His His His Pro Met Ser Asp Tyr Asp Ile Pro
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Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Gly Thr Val Pro
20 25 30

Val Glu Lys Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
35 40 45

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
50 55 60

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
65 70 75 80

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
85 90 95

Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
100 105 110

Met Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
115 120 125

Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
130 135 140

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
145 150 155 160

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
165 170 175

Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
180 185 190

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
195 200 205

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
210 215 220

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
225 230 235 240

Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
245 250 255

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Arg
260 265 270

Arg Gln Ala Cys Gly Arg Thr Arg Ala Pro Pro Pro Pro Pro Leu Arg
275 280 285

Ser Gly Cys
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<212> DNA
<213> Artificial sequence

<220>
<223> Frame adapter

<400> 13

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